

Bhattacharya, S. et al. American journal of respiratory cell and molecular biology 2009 40:359-67; Spira, A. et al. American journal of respiratory cell and molecular biology 2004 31: 601-10; Wang, L.-M. et al. American journal of respiratory and critical care medicine 2008 177:402-11). Five additional datasets were used to create query signatures of gene expression changes associated with TGF $\beta$  treatment (Malizia, A. P. et al. American journal of physiology. Lung cellular and molecular physiology 2008 295:L451-60; Clasen, S. et al. Journal of immunology 2007 178:6931-40; Koinuma, D. et al. Molecular and cellular biology 2009 29:172-86; Qin, H. et al. BMC systems biology 2009 3:73; Renzoni, E. et al. Respiratory research 2004 5:24).

**[0388]** Among the CMap data, gene expression changes resulting from treatment with the tripeptide GHK were anti-correlated with expression patterns associated with increasing regional emphysema severity ( $p=0.006$ ) and the COPD-related expression patterns observed in Bhattacharya et al., (American journal of respiratory cell and molecular biology 2009 40:359-67) and Goplon et al., (American journal of respiratory cell and molecular biology 2004 31:595-600). In Spira et al. (American journal of respiratory cell and molecular biology 2004 31: 601-10), the gene expression changes induced by GHK were anti-correlated with those associated with FEV1/FVC ( $p=0.0002$ ) but positively correlated to those that change between cases and controls or with DLCO ( $p<0.05$ ). In addition, GHK-treatment resulted in similar patterns of gene expression to those observed after TGF $\beta$  treatment of cell lines by Malizia et al., (American journal of physiology. Lung cellular and molecular physiology 2008 295:L451-60) ( $p=0.004$ ).

**[0389]** As the effects of GHK on gene-expression reported in CMap were measured in cancer cell lines, GHK treatment was verified in human lung fibroblasts. HFL-1 cultures were treated with GHK at two concentrations or with TGF $\beta$ 1. Gene expression profiling of these cells demonstrated that the 200 genes most induced by GHK at 1  $\mu$ M in cancer cell lines in the CMap dataset were enriched among genes that increase with treatment of GHK at 0.1 nM in HFL-1 cultures by GSEA (FIG. 7B). Furthermore, genes whose expression is decreased with increasing emphysema severity are enriched among genes induced by GHK at 10 nM (data not shown). Similarly, genes that increase after treatment of GHK at either concentration are enriched among genes whose expression decreases with increasing emphysema (data not shown). Genes whose expression is altered by GHK treatment at either concentration are also enriched among genes that change with TGF $\beta$ 1 treatment (FIGS. 7B-7D and data not shown). The expression profile for ITGB1, an integrin important for fibroblast migration and adhesion, was significantly down-regulated with increasing regional emphysema severity ( $p=0.0008$ ) in lung

tissue and significantly upregulated with treatment of GHK at 0.1 nM in the HFL-1 ( $p=0.004$ ). The protein levels of ITGB1 were also significantly induced with treatment of GHK, TGF $\beta$ 1, or GHK in combination with TGF $\beta$ 1 suggesting that GHK can modulate repair processes (FIGS. 8A-8B).

#### Example 5

#### Discussion

**[0390]** By measuring gene expression from regions of varying emphysema severity within the same lung, the effects of systemic differences between individuals are minimized. Herein, by using a morphologic measurement of airspace size (Lm) which reflects the degree of alveolar destruction, the gene expression changes observed were specifically related to the emphysematous component of COPD. While HRCT scans are currently the standard method for grading the severity of emphysema both within and between individuals, a close relationship between emphysema as measured by HRCT scans and Lm as measured by micro-CT has been previously reported (Hogg, et al., Proc Am Thorac Soc 2009 6:546-9). The inventors' analysis of 8 specimens per lung representing different degrees of emphysema from each individual increased the power to detect gene expression changes associated with regional emphysema severity and relate these to gene expression differences that have been observed between individuals with varying degrees of COPD and/or emphysema.

**[0391]** Identified herein are genes whose expression change as a function of regional emphysema severity. Herein, the inventors have demonstrated that progressive emphysematous destruction in COPD is associated with the down-regulation of genes involved in or downstream of tissue remodeling and wound repair pathways, suggesting a role for defects in ECM homeostasis and angiogenesis in the emphysematous destruction that occurs in association with chronic inflammation in COPD.

**[0392]** Also provided herein is a compound, GHK, which significantly reverses gene expression patterns associated with increasing emphysema severity and with increasing COPD severity. GHK treatment also induced a pattern of gene expression similar to that resulting from TGF $\beta$  pathway activation. These findings were replicated in human lung fibroblasts. In addition, the protein level of  $\beta$ 1-integrin was increased with GHK treatment. Fibroblasts treated with GHK in combination with TGF $\beta$ 1 produced significantly higher levels of  $\beta$ 1-integrin compared to fibroblasts treated with TGF $\beta$ 1 alone ( $p<0.01$ ).

**[0393]** All references described herein are incorporated herein by reference.

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#### SEQUENCE LISTING

The patent application contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (<http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US20140199411A1>). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

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